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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 35/76, C12N 7/01 // 15/86, A61K 48/00		A1	(11) International Publication Number: WO 98/37905 (43) International Publication Date: 3 September 1998 (03.09.98)
<p>(21) International Application Number: PCT/GB98/00559</p> <p>(22) International Filing Date: 24 February 1998 (24.02.98)</p> <p>(30) Priority Data: 9704046.3 27 February 1997 (27.02.97) GB</p> <p>(71) Applicant (for all designated States except US): UNIVERSITY OF LEEDS [GB/GB]; Leeds LS2 9JT (GB).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): MARKHAM, Alexander, Fred [GB/GB]; The University of Leeds, Molecular Medicine, West Riding Medical Research Unit, Clinical Sciences Building, St. James's University Building, Leeds LS9 7TF (GB). MEREDITH, David, Mark [GB/GB]; The University of Leeds, Molecular Medicine, West Riding Medical Research Unit, Clinical Sciences Building, St. James's University Building, Leeds LS9 7TF (GB).</p> <p>(74) Agent: MARKGRAAF PATENTS LIMITED; The Crescent, 54 Blossom Street, York YO2 2AP (GB).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: ARRESTABLE THERAPEUTIC VIRAL AGENT</p> <p>(57) Abstract</p> <p>The invention relates to the use of anti-herpetic sensitive viruses in the treatment of diseases and particularly cancer. More specifically, the invention concerns the genetic manipulation of viruses that are susceptible to anti-herpetic agents so as to enhance their sensitivity to said agents and/or reduce their pathogenicity.</p>			

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ARRESTABLE THERAPEUTIC VIRAL AGENT

The invention relates to a method of virus manipulation; means therefor and
5 products thereof which have particular, but not exclusive, application in
therapy/vaccine development.

Current approaches to cancer therapy rely on the surgical removal,
immunological destruction or targeted cytotoxicity of tumour tissue and/or cells.

10 Most procedures currently in use are either physically invasive or have toxic
side effects. A new form of therapy which is capable of reducing tumour mass
without these problems would make a major impact on all aspects of cancer
therapy.

15 Many animal viruses replicate in tissue culture cell lines derived from human
tumours, with the concomitant destruction of the cultured cells. These cell lines
may often be derived from cell types which would not normally support the
replication of the virus *in vivo*. The difference is often due to the high
metabolic activity of the cell line, in comparison with the resting or post-mitotic
20 form which the virus might encounter *in vivo*. Furthermore tumour cells often
demonstrate less regulated transcription patterns which may assist virus
replication through the provision of transcriptional control proteins which may
not normally exist in the differentiated cell.

25 Whereas these observations have accumulated in the scientific literature, viruses
have not, until recently, been considered of use in cancer therapy, mainly
because of the fear that virus replication would be uncontrolled and thus

become potentially lethal to this host.

Retrovirus vectors which notably are replication-defective, and which express the human tumour suppressor gene P53 have been demonstrated to have 5 efficacy in the therapy of primary lung cancer, causing either a halt in tumour growth or reduction in tumour size (1). These viruses halt the rapid growth of the tumour and presumably make it more susceptible to either committing programmed cell death (apoptosis) or stopping cell division.

10 Unfortunately many patients present with tumours which are well advanced, and are suffering secondary effects of tumour burden. Attempts are currently underway to develop a virus vector for the destruction of brain tumours, by the deletion of genes responsible for neuropathogenesis (2). Such viruses show promise in the selective destruction of rapidly growing tumour cells, whilst not 15 replicating in normal neural tissue.

A major problem with the replication-competent viruses is that they may continue to grow in the patient after tumour destruction and cause other disease symptoms, particularly if they are derived from relatively low passage, virulent 20 virus strains. Furthermore, the innate host immunity to these viruses may inhibit virus spread and destroy the infected tumour cells.

We have, contrary to prevailing wisdom and generally accepted practices in the art of cancer therapy, used replication-competent animal viruses in a most 25 elegant way. We have exploited the phenomenon that all herpesviruses are variably susceptible to anti-herpetic drugs/agents as the premise of the invention. Additionally we have manipulated viruses in a unique manner so as

to destroy/delete/deactivate selected genes associated with pathogenicity whilst optionally manipulating said viruses to achieve enhanced sensitivity to anti-herpetic drugs/agents. Consequently we have advantageously produced a replication-competent animal virus that is apathogenic to man and that can 5 easily be arrested, either temporarily or permanently, at any time in the course of therapy.

Furthermore, anti-herpetic drugs are known to have negligible side effects even with repeated and long term dosing, and certainly represent a class of drugs with 10 less serious side effects than most traditional chemotherapeutic agents. Chemotherapeutic agents have significant toxic side effects upon repeated long term administration including hair loss, therefore the former class of drugs represent a means of reducing patient discomfort of both a physical and psychological nature.

15 In addition, the viral agents as hereinbefore described are particularly suitable for use in gene therapy in humans and animals because it is well known that the viruses are susceptible to anti-herpetic drugs such as acyclovir. Thus, in the instance where one wanted to terminate, either temporarily or permanently, a 20 gene therapy treatment, one could simply administer acyclovir to an individual receiving such gene therapy and this would have the effect of destroying the gene therapy vector, and the cells supporting vector replication, thus terminating the therapy. The ability to control the gene therapy treatment once it has commenced is of particular importance in the context of treating children or 25 adults who are capable of reproduction because it enables a gene therapy regime to be terminated before the individual reproduces and it therefore prevents the gene of the relevant treatment being transmitted in the germ cell line and so

being passed on through generations. It is also of particular note that it is possible to engineer the gene therapy vector so as to make it particularly sensitive to anti-herpetic drugs such as acyclovir. This can be undertaken using conventional techniques by, for example, the insertion of a herpes simplex 5 virus thymidine kinase gene in the relevant vector. The aforementioned enzyme phosphorylates acyclovir and so renders the virus particularly sensitive to its effects.

The invention claimed in this application is the novel use of animal viruses as 10 arrestable therapeutic agents and optionally the introduction of a heterologous gene which make the virus hypersensitive to treatment with anti-herpetic drugs/agents, thus inventively permitting complete, effective, safe and accurate termination of therapy with minimal side effects to a patient.

15 It is a first object of the invention to provide an arrestable therapeutic viral agent derived from an animal virus which intrinsically, or following suitable manipulation, is susceptible to anti-herpetic drugs/agents.

It is a further object of the invention to provide an arrestable therapeutic viral 20 agent which comprises a replication-competent virus vector for tumour destruction.

It is a yet further object of the invention to provide an arrestable therapeutic viral agent that is susceptible to elimination from the host/patient using anti-25 herpetic agents.

It is a yet further object of the invention to minimise the side-effects to the

host/patient during cancer therapy treatment.

It is a yet further object of the invention to provide a therapy treatment that can be easily terminated either temporarily or permanently.

5

According to a first aspect of the invention there is provided use of an arrestable therapeutic viral agent that is susceptible to elimination from a host/patient using anti-herpetic agents and that is capable of replicating in human tissue for treating tumours.

10

Reference herein to tumours is intended to include any neoplasm, malignant growth, cancer or morbid enlargement in any human tissue of a solid nature or otherwise.

15

Reference herein to arrestable is intended to include either temporary or permanent arrest of viral activity.

Reference herein to elimination is intended to include short, medium or long term elimination, typically characterised by cessation of virus replication.

20

Reference herein to anti-herpetic drugs/agents is intended to include reference to any known agent that has been demonstrated to have anti-herpetic activity and is intended to include analogues and homologues thereof.

25

In a yet further preferred embodiment of the invention there is provided use of a herpetic virus capable of replicating in human tissue ideally for use in treating tumours.

Ideally said herpetic virus is engineered or altered so as to exhibit enhanced sensitivity to said anti-herpetic agents.

5 Reference herein to herpetic virus is intended to include reference to any virus that is intrinsically sensitive to or suitably altered so as to be sensitive to anti-herpetic drugs/agents such as acyclovir and/or pencyclovir and/or homologues and/or analogues thereof including other related drugs/agents such as those typically known as orphan drugs.

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In preferred embodiments of the invention we have selected two herpes viruses, human herpes simplex virus type 1(HSV-1) and equine herpesvirus type 1 (EHV-1) as potential arrestable therapeutics.

15 EHV-1 has been chosen as it is one of the few herpesviruses whose natural route of infection is at mucosal surfaces, additionally the virus replicates in cell lines derived from a wide range of human tumours, but does not cause diseases in humans (3). The virus is much more stable in such an environment than other herpesviruses. EHV-1 is not a human pathogen and there is no innate
20 immune response to this virus in the population. Thus the virus would not be inhibited from spreading through a tumour on primary inoculation, but the infected cells would not represent (at least to start with) targets for host destruction.

25 Both the aforementioned viruses replicate in a wide range of human cell types in culture, destroying their host cell. All herpesviruses are variably susceptible to inhibition by acyclovir. An additional advantage of both viruses is that they

maybe engineered to replicate only in dividing cells.

However HSV-1 is a human pathogen; >90% of the population have an innate immune response to the virus. Thus tumour cells infected with this virus would
5 be recognised by the host immune system and targeted for destruction. It is possible, though, that the innate response may prevent the virus spreading through a tumour efficiently.

In a yet further preferred embodiment of the invention there is provided an
10 herpes virus capable of replicating in human tissue ideally for use in treating tumours, and more ideally said herpesvirus is a herpes simplex virus and most ideally said herpes simplex virus is HSV-1 strain HFEM.

In a yet further preferred embodiment of the invention there is provided use of
15 an equine herpesvirus capable of replicating in human tissue ideally for use in treating tumours.

Ideally said equine herpesvirus is engineered or altered so as to exhibit enhanced sensitivity to said anti-herpetic agent.

20

We know, preferably, since the virus gene product responsible for activating acyclovir, TK, is expressed at a lower level in EHV-1 than HSV-1, thus making the virus less susceptible to the antiviral drug, we can replace this gene with that derived from HSV-1 to inventively improve the sensitivity to acyclovir.

25

In addition we are deleting three genes which are known to be important for EHV-1 pathogenicity of the disease in small animal models. This will ensure

that the virus is considered by regulatory authorities to be a severely disabled, replication-competent virus which does not need to be handled under a high level of containment, which might otherwise be a problem when introducing a heterologous gene into an otherwise wild-type virus. Thus we have invented a 5 novel approach to overcoming problems associated with the prior art.

In a yet further preferred embodiment of the invention any of the aforementioned said viruses lacks or has at least one mutation in at least one gene responsible for, or associated with, pathogenicity in humans, such that the 10 gene product is lacking or is non-functional with respect to human pathogenicity.

Thus any of the aforementioned viruses have been genetically manipulated so as to remove genes which are known to be determinants of pathogenicity 15 typically in animal models. The agent derived from HSV-1 is based on a laboratory-adapted strain of the virus which has a spontaneous deletion in the genome and affects a gene responsible for the spread of the virus from peripheral sites (4). Additional mutations which are known to affect pathogenicity will be introduced into this virus.

20 Optionally, as EHV-1 is apparently apathogenic for humans, there may be no need for gene deletion.

25 In a yet further preferred embodiment of the invention there is provided an animal virus modified so that at least one gene relating to pathogenicity is/are mutated and/or deleted and/or disabled.

Thus in a yet further preferred embodiment of the invention there is provided an herpesvirus, as hereinbefore described, modified so that at least one gene relating to pathogenicity is/are mutated and/or deleted and/or disabled, ideally said herpesvirus is HSV-1 strain HFEM and most ideally said gene 5 determinant(s) is/are U13 and/or UL50.

Thus is a yet further preferred embodiment of the invention there is provided an equine herpesvirus, as hereinbefore described, modified so that at least one gene relating to pathogenicity is/are mutated and/or deleted and/or disabled, ideally 10 said gene determinant(s) is/are gene 13 and/or gene 49 and/or gene 9.

According to a second aspect of the invention there is provided a modified acyclovir-sensitive animal virus that is replication-competent but has been genetically altered so that it exhibits enhanced sensitivity to acyclovir or 15 homologues or analogues thereof.

In a preferred embodiment of the invention there is provided a modified acyclovir-sensitive herpesvirus that is replication competent but has been genetically altered so that it exhibits enhanced sensitivity to acyclovir or 20 homologues or analogues thereof.

In a yet further preferred embodiment of the invention there is provided a modified equine herpesvirus that is replication-competent but has been genetically altered so that it exhibits enhanced sensitivity to acyclovir or 25 homologues or analogues thereof.

In a yet further preferred embodiment of the invention there is provided an

animal virus modified so as to carry a selected gene or agent such as a drug for use in therapy, ideally said animal virus comprises an herpesvirus and more ideally said animal virus comprises an equine herpesvirus.

5 According to a third aspect of the invention there is provided a pharmaceutical composition comprising either one or more of said aforementioned viruses, optionally in combination with a suitable carrier, ideally said carrier comprises a solution or suspension or the like, the nature of which is not intended to limit the scope of the application.

According to a fourth aspect of the invention there is provided a method of treating an individual, wherein said individual is identified as having or likely to have a tumour, comprising:-

15 I administering the aforementioned pharmaceutical composition to said individual to be treated, ideally the route of administration is by injection and most ideally injection directly to a tumour site and/or its immediate environs; and

20 II optionally, selectively terminating or abating treatment with said pharmaceutical composition by administering to said individual an effective amount of an anti-viral agent, ideally the route of administration of said anti-viral agent is by injection and/or oral and most ideally said anti-viral agent is an anti-herpetic agent such as 25 acyclovir and/or homologues and/or analogues thereof.

Methods

Inactivation of the EHV-1 thymidine kinase gene and production of a new strain of EHV-1 incorporating the thymidine kinase gene from herpes simplex virus type 1.

The EHV-1 TK gene is situated between coordinates 69910 and 70968 in the published sequence of virus strain Ab4. The virus strain to be manipulated was Ab1, isolated at exactly the same time as Ab4 and has an essentially identical genome sequence (5). The DNA sequence between coordinates 69910 and 71200 was amplified by PCR and the 1290bp fragment was cloned into the plasmid LITMUS 38. This plasmid termed pDM551 was digested with the restriction endonuclease Fsp 1 to remove a 315bp fragment of the TK gene coding sequence. The plasmid was then religated to create pDM552. This construct was transfected into BHK cells with purified infectious EHV-1 DNA using standard procedures. Cytopathic effect was allowed to proceed until the majority of the cell sheet was destroyed by infectious virus. Cells were then scraped from the dish, disrupted by ultrasonic vibration and the titre of the virus stock determined, in the presence and absence of 100µg/ml acyclovir, which estimated the percentage of the virus population resistant to acyclovir, i.e. those that were now TK negative. The virus stock was replated using limiting dilution methods in 96 well plates in tissue culture medium containing acyclovir. Individual plaque isolates of acyclovir-resistant viruses were then tested using PCR for the presence of the engineered TK deletion. Stocks of this virus were then plaque purified and designated as Ab1_38.

The HSV-1 TK gene (UL23) is located between nucleotides 46674 and 47802

in the published sequence of HSV-1 strain 17. The complete coding sequence was amplified by PCR and cloned into the pCMVbeta, from which the beta-galactosidase gene had been removed by digestion with the restriction endonuclease Not 1, generating pDM553. An expression cassette consisting of 5 the TK gene under the control of the CMV promoter was removed from this plasmid using restriction endonucleases Eco R1 and Sal 1, and ligated into the Fsp 1 site of pDM552, to create plasmid pDM554. Infectious virus DNA prepared from AB4_38 was cotransfected with DNA from pDM552 and progeny virus isolated and titrated. Individual virus stocks were isolated 10 through plating at limiting dilution, and then replica plated into duplicate 96 well plates, one in the presence of 100µg/ml acyclovir. Viruses which were now susceptible to acyclovir (i.e. were recombinants expressing HSV-1 TK), were removed from the replica plate grown in the absence of acyclovir, and subjected to 2 rounds of further plaque purification. Genome integrity was 15 confirmed by Southern blotting, and the derivative viruses were termed AB1RTK1.

HSV-1 gene UL13

20 The UL13 gene is located between coordinates 26950 and 28504 in the published sequence of HSV-1 strain 17. The complete gene was available in this laboratory in a plasmid designated pYW101 which contains a DNA fragment derived from HSV-1 strain 17 between coordinates 29336 and 25149. A fragment of this was derived by digestion with Dde 1 and cloned into the 25 Sma 1 site of pUC19. This plasmid was termed pYW201. Further digestion of this plasmid with Hind III removed a 52bp fragment from the UL13 coding sequence, the plasmid was religated and termed pYW211. This plasmid was

then redigested with HindII and a cassette containing the beta-galactoside gene under control of the CMVIE3 promoter was cloned into the plasmid to derive pYW212. Infectious virus DNA was transfected with pYW212 exactly as aforescribed and cytopathic effect allowed to proceed. Progeny virus was 5 isolated, diluted and plated out on fresh monolayers of BHK cells in the presence of the beta-galactoside colourimetric, X-gal. Virus plaques which turned blue were picked and recombinant virus was isolated in a clonal form through 3 rounds of plaque purification. Genome integrity was confirmed by Southern blotting.

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HSV-1 gene UL50

The HSV-1 UL50 gene is located between coordinates 107,010 and 108,123 in the published sequence of HSV-1 strain 17. This DNA sequence as above was 15 amplified by PCR and the 1114 base pair fragment was cloned into plasmid LITMUS 38. The resulting plasmid, designated pDM710, was digested with the restriction endonuclease Sma 1 to release a 96 bp and a 47bp fragment. The remaining plasmid was made blunt ended with T4 DNA polymerase and a cassette of the beta-galactosidase gene under control of the CMVIE3 promoter 20 was ligated into site to create pDM711. Infectious virus DNA was transfected with pDM711 exactly as described in the previous section and cytopathic effect allowed to proceed. Progeny virus was isolated, diluted and plated out on fresh monolayers of BHK cells in the presence of the beta- galactosidase colourimetric indicator, X-gal. Virus plaques which turned blue were picked 25 and recombinant virus was isolated in a clonal form through 3 rounds of plaque purification. Genome integrity was confirmed by Southern blotting.

EHV-1 gene 13

EHV-1 gene 13 is located between coordinates 15317 and 17932 in the published sequence of EHV-1 strain Ab4. This DNA sequence (from strain 5 Ab1 as above was amplified by PCR and the 2640 base pair fragment was cloned into plasmid LITMUS 38. The resulting plasmid, designated pDM570, was digested with the restriction endonucleases Sma 1 and Bam H1 to release a 1333bp fragment. The remaining plasmid was made blunt ended with T4 10 DNA polymerase and a cassette of the beta-galactosidase gene under control of the CMVIE3 promoter was ligated into the site to create pDM571. Infectious virus DNA was transfected with pDM571 exactly as described in the previous section and cytopathic effect allowed to proceed. Progeny virus was isolated, diluted and plated out on fresh monolayers of BHK cells in the presence of the beta-galactosidase colourimetric indicator, X-gal. Virus plaques which turned 15 blue were picked and recombinant virus was isolated in a clonal form through 3 rounds of plaque purification. Genome integrity was confirmed by Southern blotting.

Deletion of gene 49, virion protein kinase

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The gene encoding the virion protein kinase is located between genome coordinates 89369 and 91153. The complete coding sequence was amplified by PCR from purified virus DNA as template to generate a 1782 bp fragment. This was cloned into the plasmid LITMUS38 to create plasmid pDM561. This was 25 then digested with the restriction endonucleases Nru1 and Pml 1 to release a 644bp fragment. The plasmid was then made blunt ended and a cassette of the beta-galactosidase gene under control of the CMV IE3 promoter was ligated

into the site to generate pDM562. Infectious virus DNA was co-transfected with plasmid pDM562 into BHK cells, and cytopathic effect allowed to proceed. Progeny virus was harvested, diluted and plated out onto fresh monolayers of BHK cells in the presence of the beta-galactosidase colorimetric 5 indicator, X-gal. Virus plaques which turned blue were picked and the recombinant virus was isolated in a clonal form through several rounds of plaque purification. Genome integrity was confirmed by Southern blotting.

EVH-1 gene 9

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EVH-1 gene 9 is located between coordinates 11,135 and 12,115 in the published sequence of EHV-1 strain Ab4. This DNA sequence (from strain Ab1 as above) was amplified by PCR and the 980 base pair fragment was cloned into the plasmid LITMUS 38. The resulting plasmid, designated 15 pDM580, was designated with the restriction endonucleases Pml 1 and Bsp M1 to release a 377bp fragment. The remaining plasmid was made blunt ended with T4 DNA polymerase and a cassette of the beta-galactosidase gene under control of the CMVIE3 promoter was ligated into the site to create pDM581. Infectious virus DNA was transfected with pDM581 exactly as described in the 20 previous section and cytopathic effect allowed to proceed. Progeny virus was isolated, diluted and plated out on fresh monolayers of BHK cells in the presence of the beta galactosidase colourimetric indicator, X-gal. Virus plaques which turned blue were picked and recombinant virus was isolated in a clonal form through 3 rounds of plaque purification. Genome integrity was confirmed 25 by Southern blotting.

Construction of a Recombination Cassette, to Create a Strain of EHV-1 Containing the HSV-1 TK Gene.

5 The plasmid pHSV-106 (Life Science Technologies) was digested with BamH1 to produce a 3.4Kbp fragment containing the complete HSV-1 TK gene.

Two sets of PCR primers flanking the EHV-1 TK gene (gene 38) were designed to include appropriate restriction enzyme sites to ligate to either end of the 10 BamH1 TK fragment. The 5' flanking region encompasses 404bp, the 3' flanking region encompassed 1,002bp flanking sequences were ligated to the BamH1 fragment, and cloned into pUC18. Clones were screened for the presence of DNA sequences in the correct orientation, and a typical clone was named p38TK.

15 Purified p38TK was co-transfected with purified EHV-1 DNA, and recombinant virus was isolated using standard procedures.

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CLAIMS

- 5 1. Use of an arrestable therapeutic viral agent, that is susceptible to elimination from a host/patient using an anti-herpetic agent and that is capable of replicating in human tissue, for treating tumours.
- 10 2. Use according to Claim 1 wherein said virus is a herpetic virus capable of replicating in human tissue.
3. Use according to any preceding claim wherein said agent is a α herpes virus.
- 15 4. Use according to any preceding claim wherein said agent is a herpes simplex virus.
5. Use according to Claim 4 wherein said agent is human herpes simplex virus type 1 (HSV-1).
- 20 6. Use according to Claim 5 wherein said agent is HSV-1 strain HSEM.
7. Use according to Claims 1-3 wherein said agent is equine herpes virus type 1 (EHV-1).
- 25 8. Use according to any preceding Claim wherein said virus has been recombinantly engineered or altered so as to exhibit enhanced sensitivity to said anti-herpetic agent.
9. Use according to Claim 8 wherein said engineering involves the insertion of a herpes simplex virus thymidine kinase gene into said viral agent.

10. Use according to any preceding claim wherein said agent has been recombinantly engineered to replicate only in dividing cells.

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11. Use of an equine herpes virus capable of replicating in human tissue for treating tumours.

12. Use according to Claim 11 wherein said equine herpes virus has been engineered 10 or altered so as to exhibit enhanced sensitivity to an anti-herpetic agent whereby said equine herpes virus can be eliminated from a host/patient.

13. Use according to Claim 12 wherein said engineering involves the insertion of a herpes simplex virus thymidine kinase gene into said equine herpes virus.

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14. Use according to any preceding claim wherein said virus lacks, or has at least one mutation, in at least one gene responsible for, or associated with, pathogenicity in humans, such that the gene product is lacking or is non-functional with respect to human pathogenicity.

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15. Use according to Claim 14 wherein said pathogenic gene is mutated and/or deleted and/or disabled.

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16. Use according to Claims 14 or 15, when dependent upon Claims 5 or 6, wherein said pathogenic gene is U13 or UL15.

17. Use according to Claims 14 or 15, when dependent upon Claim 7, wherein said pathogenic gene is gene 13 and/or gene 49 and/or gene 9.

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18. A modified, anti-herpetic sensitive, animal virus that is replication-competent but that has been genetically altered so that it exhibits enhanced sensitivity to anti-herpetic agents or homologues or analogues thereof.
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19. A virus according to Claim 18 wherein said anti-herpetic agent is acyclovir.
20. A virus according to Claim 18 or 19 wherein said alteration involves the insertion
10 of a thymidine kinase gene into said virus.
21. A virus according to Claims 18, 19 or 20 wherein said virus is an α herpes virus.
22. A virus according to Claim 21 wherein said virus is an equine herpes virus.
15
23. A virus according to Claims 18-22 wherein said virus has been modified so as to carry a selected gene or agent such as a drug for use in therapy.
24. A pharmaceutical composition comprising either one or more of the viruses
20 according to Claims 18-23, optionally, in combination with a suitable carrier.
25. A method of treating an individual, wherein said individual is identified as having, or likely to have, a tumour, comprising
25
 - i) administering a pharmaceutical composition according to Claim 24 to said individual to be treated; and
 - ii) optionally, selectively terminating or abating treatment with said pharmaceutical composition by administering to said individual an effective amount of an anti-herpetic agent.
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26. A method according to Claim 25 wherein said pharmaceutical composition is injected into the site to be treated.

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27. A method according to Claims 25 and 26 wherein said anti-viral agent is injected into the site to be treated

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INTERNATIONAL SEARCH REPORT

Internal ref Application No
PCT/GB 98/00559

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K35/76 C12N7/01 //C12N15/86, A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^o	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 04804 A (AMERICAN CYANAMID COMPANY) 13 February 1997 see page 6, line 15 - line 27; claims 1,2,5-12 see page 17, line 3 - line 15 see page 18, line 4 - line 7 ---	1-6,10, 14,15
X	WO 96 00007 A (GEORGETOWN UNIVERSITY) 4 January 1996 see page 1, line 1 - line 12; claims 1-12 see page 8, line 26 - page 9, line 18 see page 15, line 36 - page 18, line 13; examples 1,2 ---	1-6,8, 10,14,15

Further documents are listed in the continuation of box C.

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

12 June 1998

Date of mailing of the International search report

15.07.1998

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INTERNATIONAL SEARCH REPORT

Internat	al Application No
PCT/GB	98/00559

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	T. MINETA ET AL.: "ATTENUATED MULTI-MUTATED HERPES SIMPLEX VIRUS-1 FOR THE TREATMENT OF MALIGNANT GLIOMAS." NATURE MEDICINE., vol. 1, no. 9, September 1995, NEW YORK, NY., US, pages 938-943, XP002067917 see the whole document ---	1-6,8, 10,14,15
X	T. MINETA ET AL.: "TREATMENT OF MALIGNANT GLIOMAS USING GANCICLOVIR-HYPERSENSITIVE, RIBONUCLEOTIDE REDUCTASE-DEFICIENT HERPES SIMPLEX VIRAL MUTANT." CANCER RESEARCH, vol. 54, 1 August 1994, BALTIMORE, MD, US, pages 3963-3966, XP002067918 see page 3963, left-hand column, paragraph 2 - right-hand column, line 20 see page 3965, right-hand column, line 3 - line 11 ---	1-6,8, 10,14,15
X	J.M. MARKERT ET AL.: "REDUCTION AND ELIMINATION OF ENCEPHALITIS IN AN EXPERIMENTAL GLIOMATHERAPY MODEL WITH ATTENUATED HERPES SIMPLEX MUTANTS THAT RETAIN SUSCEPTIBILITY TO ACYCLOVIR" NEUROSURGERY, vol. 32, no. 4, 1 April 1993, BALTIMORE, MD, US, pages 597-603, XP000561128 see the whole document ---	1-6,10, 14,15
A	EP 0 668 355 A (AKZO NOBEL N.V.) 23 August 1995 see page 3, line 39 - line 42; claims -----	7,14,15, 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 98/00559

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-17 and 25-27 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern	ai Application No
	PCT/GB 98/00559

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9704804	A 13-02-1997	AU 6713996	A	26-02-1997
WO 9600007	A 04-01-1996	US 5585096	A	17-12-1996
		AU 679644	B	03-07-1997
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		CA 2193491	A	04-01-1996
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		JP 10501990	T	24-02-1998
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EP 668355	A 23-08-1995	JP 7236489	A	12-09-1995
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